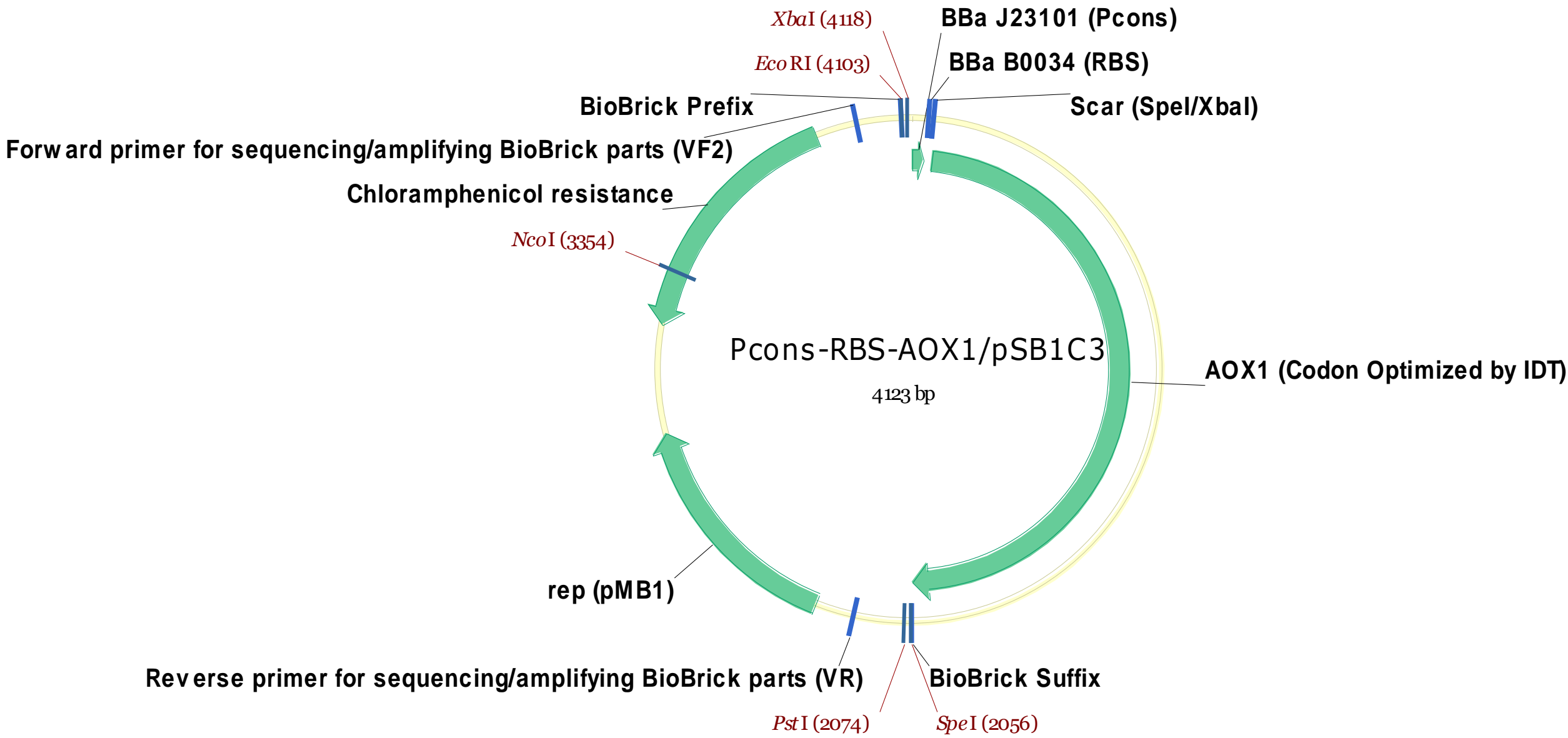


**Pcons-RBS-
AOX1/pSB1C3**



Strategy

- Vector: AOX1/pSB1C3 (4060 bp) → EcoRI + XbaI
- Insert: EcoRI-XbaI-Pcons-RBS-SpeI amplified by PCR → EcoRI + SpeI
 - Template: NCTU #3_BBak_K1694035_pSB1C3 (#3) Pcons-RBS-Lpp-OmpA-NcoI/pSB1C3
 - PCR product size: ~83 bp

Primer Design

- Pcons-XbaI-EcoRI-F:

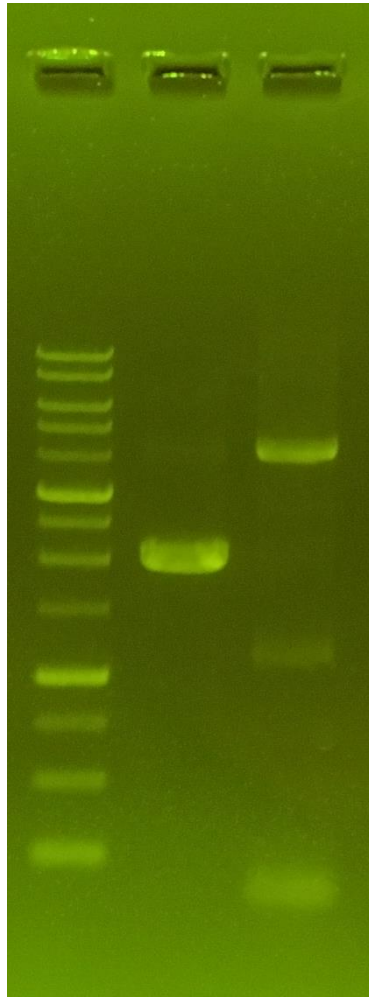
- 5'-

- AGTACAGAATTCGCGGCCGCTTCTAGAGTTTACAGCTAGCTCAG -
3'

- RBS-SpeI-R: (New)

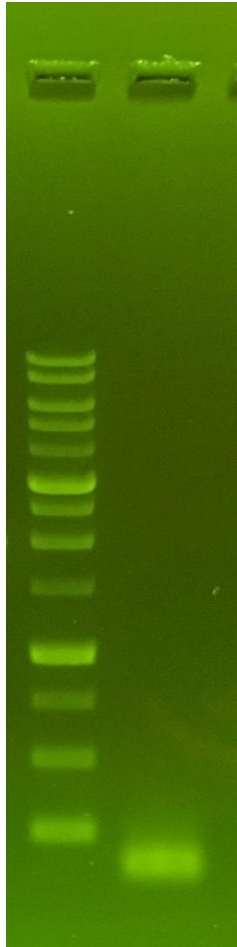
- 5'- GCGGGCACTAGTATTTCTCCTTTCTCTAGTA -3'

PCR for Pcons-RBS with KOD-plus



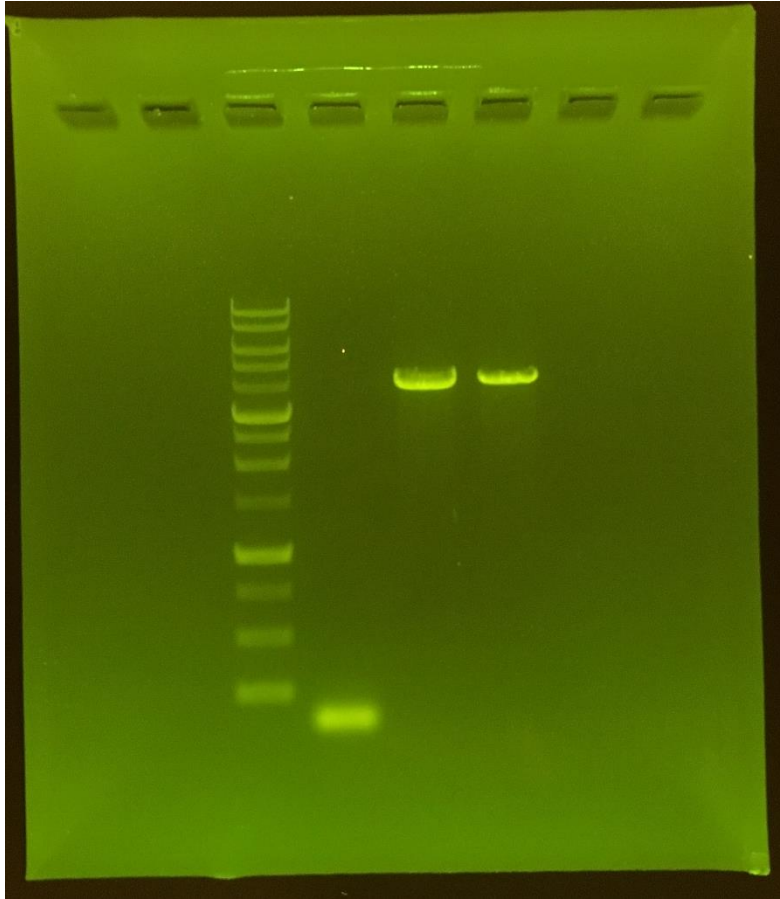
- Date: 2016.09.05
- Lane:
 1. XbaI-AOX1-PstI
 2. EcoRI-Pcons-RBS-SpeI

Touch Down PCR for Pcons-RBS with KOD-plus



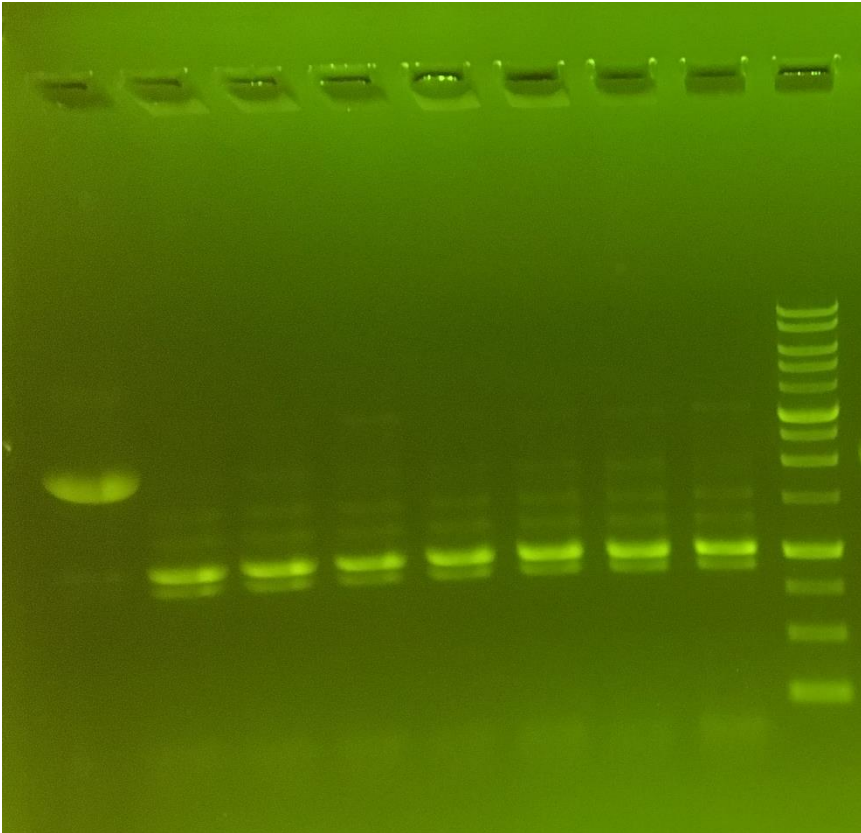
- Lane:
 1. EcoRI-Pcons-RBS-SpeI (~100bp)

Ligation



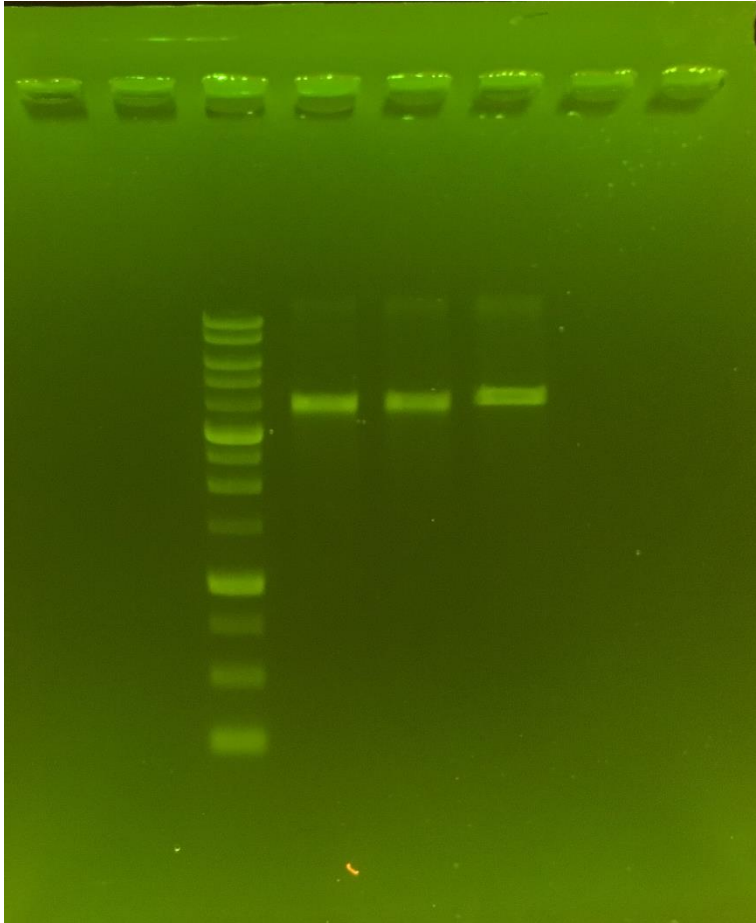
- Date: 2016.09.22
- Lane:
 1. (Insert) Pcons-RBS: EcoRI + SpeI
 2. (Vector) AOX1/pSB1C3 #1: EcoRI + XbaI
 3. (Vector) AOX2/pSB1C3 #2-7: EcoRI + XbaI

Colony PCR



- Date: 2016.09.23
- Clone: Pcons-RBS-AOX1/pSB1C3
- Primer:
 1. Pcons-XbaI-EcoRI-F
 2. AOX1-PstI-R
- Lane: #1-1 ~ #1-8
- #1-1 → Plasmid extraction → RE check

RE check



- Date: 2016.09.30
- RE: XbaI
- Lane:
 1. Pcons-RBS-AOX1/pSB1C3 #1-1
 2. Pcons-RBS-AOX2/pSB1C3 #2-2
 3. Pcons-RBS-AOX2/pSB1C3 #2-3

Sequencing

- Date: 2016.10.04
- Sequencing primers: VF2
- Clone: Pcons-RBS-AOX1/pSB1C3 #1-1
- File: “2016.10.04_Pcons-RBS-AOX1, 2_pSB1C3 (#1-1, #2-2, #2-3)”
- Result: revised → make a stock